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Appraisal of oxidative stress in Pea (*Pisum sativum* L.) in response to different insecticides against *Helicoverpa armigera L*

Ahmad Raza[1], Muhammad Mubashar Zafar[2], Zafar Hussain[2], Sohail Ahmed[1], Muhammad Saqib Mushtaq[3], Abd-ul-Rahman[1]

1.Department of Entomology, University of Agriculture Faisalabad, Pakistan
2.Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
3.Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan

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ABSTRACT

Pea (*Pisum sativum L.*) is the most important perishable vegetable which contains many nutrients and cultivated on large acreages all over the world. Pod borer (*Helicoverpa armigera L.*) is the important pest of pea crop. It causes heavy losses to the crop up to 80 to 90%. The most common approach to control this pest is chemical control by using different insecticides which are more effective, rapid and fruitful to manage this pest. But their excessive and uneven use cause perilous stress on plant. The present study was planned to check the efficacy of three different insecticides (Bifenthrin, Emamectin and Lambda cyhalothrin) against *H. armigera* population and examined different stress-related parameters in pea plant by studying antioxidant enzyme activities. Two local varieties Aleena Gold and Climax were sown at experimental area, Department of Entomology, University of Agriculture, Faisalabad (Pakistan) by using factorial under Randomized Complete Block Design (RCBD). A laboratory bioassay was performed to check the

mortality of *H. armigera*. The results proved that Emamectin gave more effective control on *Helicoverpa armigera* with 78% mortality while Lambdacyhalothrin gave 76% mortality and Bifenthrin gave minimum mortality i.e. 71%.The lipid peroxidation rate, proline content and activities of antioxidant enzymes CAT, SOD and POD had been examined. It was proved that all these insecticides affect the antioxidant enzymes activity and also effect the lipid peroxidation rate and proline contents in the plant. Bifenthrin was more toxic for plants and more effected the antioxidant enzymes activity. The stress was observed in all three weeks but it was maximum in 2nd week after that it became less in the 3rd week. Emamectin showed less stress as compared to Bifenthrin while Lambda cyhalothrin showed the least stress in comparison with Emamectin and Bifenthrin.

Keywords: Antioxidant, Bifenthrin, Emamectin, Lambda cyhalothrin, oxidative stress.

1. INTRODUCTION

Pea (*Pisum sativum L.*) has its place in *Leguminosae* family and is one of the world's firstborn farm crop all over the world (Ambrose, 1995; Zohary and Hopf, 2000). After soybean and common beans, peas are the 3rd highly essential legume grains in the universe (Timmerman-Vaughan *et al*., 2005). Pea cultivation is done on almost 0.73 million ha in the subcontinent and annual produce obtained is almost 0.72 million tons including 906 kg/ha an average yield of pea seeds (Singh and Sarvanan, 2011). Peas contain many nutritional constituents which have nearly totally rudimentary nutrients such as carbohydrates and proteins which are essential for our body and health. Vitamins, minerals, dietary fibers and antioxidant compounds also originate in peas in less amount (Urbano *et al.,* 2005). Although peas have great nutritional strength as an economic origin of protein, vitamins, carbohydrates, minerals and even certain microelements, thus limited use of this important legume due to some anti-nutritional facts (Gatel and Grosjean 1990). Peas have the abilities to nitrogen fixing similar to other legumes which are helpful to save fertilizers and money (Westerfield, 2013). The main big causes of its lower production are farming on marginal land, unwarranted fertilizer application and occurrence of diseases and insect pests (Zohary and Hopf, 2000).

The pod foreur (*Helicoverpa armigera*) is the secondary insect nuisibles of pea crop all over the pea cultivated regions (Anwar and Shafiq, 1993). It causes almost 30-40% pod injury to the crop (Hashmi, 1994). On semiarid crops, yield loss caused by the eruption of *H. armigera* may surpass $2 billion dollars annually, additional expenditures of chemical sprays to eradicate this pest is $500 (Sharma, 2001). In both cotton and pulses, total losses exceeded $530 million and insecticide cost to control this pest is $127.5 million on pulses and cotton, annually. In chickpea and pigeonpea, the worldwide extent of losses has been estimated $927 million annually in reason of this pest (Sharma *et al*., 2005). *Helicoverpa armigera* is an insatiable feeder pest which causes damage to more than 100 plant classes plus economically important and prevalent crops like cotton, tomato, chickpea, green pea, pigeon pea and maize etc. (Talekar *et al*. 2006). The part of preferred plants to enhance procreations of *H. armigera*is luminous and comprise. The accessibility of diverse preferred plants performs a crucial part to bring about population outbursts for poly feeder little creatures (Singh and Parihar 1988). Almost 180 farms grown host along with wild species including 45 plant families have been reported which are attacked by *H. armigera*, worldwide (Venette *et al*., 2003). Intensive use of broad spectrum insecticides has improved concomitant insecticide resistance, as well as regular and excess availability of food plants, have made *H. armigera* to be an important crop pest (Fathipour and Naseri 2011). The fecundity rate of *H. armigera* larvae are influenced by the various nutritive regulators of host plants and pest's population dynamics were affected (Ruan and Wu 2001). As it has diverse nature, multiple hosts, insecticidal resistance and great fecundity potential, it has attained drastic position and becoming out of control (Ahmed *et al.,* 2000). Two to three larvae on the cotton plant have the ability to destroy the entire plant within 15 days (CABI, 2007). New larvae of *H. armigera* of 2nd or 3rd instar may cause 65% less production of cotton. Many diverse conventional insecticides are used to the control of *H. armigera* which is harmful to vegetables (Wakchaure, 1998; Nadagouda *et al.*, 2010).

This pest is mainly managed by synthetic insecticides but the pest status of this insect has increased rapidly due to the spread of some species which are resistant to the conventional insecticides. This has been caused the various outbreaks of *H. armigera*in Australia, India, Pakistan and several further different areas of the den (Singh and Mulick, 2002). The wide use of pesticides at high concentration produces toxicological problems that have deleterious effects on plant growth and its vigor (Yildiztekin *et al.,* 2015). But many serious issues are raised about environmental problems, pest resurgence and secondary pest outbreaks (Breeden *et al*., 2006). Bifenthrin and cypermethrin have the great impact to control the population of pod *Helicoverpa armigera*on horticultural crops (Mujica *et al.*, 2000) and on vegetables (Civelek and Weintraub, 2003).

The other insecticides, spinosad, methomyl, and indoxacarb were considered surpass to manage this pest (Ferguson, 2004). Spinosad is more important because it has a distinctive way of action contrary to a large number of target nuisibles i.e. leaves, fruits

madens, mites, thrips, different mooches or flies. It has been reported that it is less toxic to vertebrates i.e. birds and animals (Eger and Lindenberg, 1998; Van *et al.,* 2006).

Different types of stress are produced in plants in response to insecticide application in which two main groups of stress factors are natural and anthropogenic stresses which contain heavy metals, pesticides and air pollutants (Lichtenthaler, 1996). Abiotic stresses like low temperature, UV radiation, nutrient deficiency, drought and high salinity have negative effects on reproduction, development, and growth of the plant. Plants depend on proteomic flexibility to reconstruct themselves to achieve more prospects of existence in different environments (Lee and Kim, 2011). Soil solution has been affected the plants by salt through minimum osmotic potential i.e. (osmotic stress), particular ion effects i.e. (salt stress), differences in nutritif gaining i.e. (nutritional stress), or all these three factors collectively effect on the plant (Ashraf, 2004). Extensive losses to agricultural crops are caused by abiotic stresses, at the global level (Brey *et al*., 2000). Plants have a natural anti-oxidative mechanism to prevent from hazardous effects of cellular membranes, produced by reactive oxygen species (ROS) (Zaefyzadeh *et al.*, 2009). It is studied that reactive oxygen species (ROS) are accountable in many stress influenced cellular structures. So it is extensively believed that ROS is the main cause of chemical toxicity (Nicholas and Wood, 2001; Chen *et al.*, 2010; Faize *et al.*, 2011).

The reasons behind oxidative stress are: (I) due to the imbalance of normal cell physiology causes inequity between detoxification and ROS generation; (ii) constituent part of stress signaling is ROS biosynthesis *de novo* and for adaption and defense immunity response is needed. The coexistence of these mechanisms is due to directly generating ROS (ultraviolet or ozone, transition metals) by stress factors. Moreover, it excites ROS production by peroxidases and NADPH oxidases or peroxidases (Zhang *et al*., 2010; Nawkar *et al*., 2013). One of the oldest stresses on the plant is due to the effect of oxygen derivatives (Dowling and Simmons, 2009). Numerous enzymatic and non-enzymatic activities are generated which prevent the plant from oxidative damage produced by ROS (Ashraf, 2009). Due to the stress condition, the plant may change the actions of ROS scrounging enzymes superoxidase dismutase (SOD), catalase (CAT) and peroxidase (POD) (Tuluce and Celik, 2006). SOD, CAT, POD and APX are the dominant enzymatic antioxidants while tochopherol and ascorbic acid are the major non-enzymatic antioxidants produced by the plants to overcome the adverse effects forced by ROS under the stressful conditions (Mittler, 2002).

POD is frequently dispersed in all higher plants and provides protection to the cell from damaging effects by catalyzing its putrefaction via oxidation of phenolic and indolicco-substrates (Dionisio-Sese and Tobita, 1998; Lin and Kao, 2002). The dismutation of O_2^- to H_2O_2 by catalyzing SOD is occurs. Almost all the aerobic cells have CAT in peroxisomes and practically missing in chloroplast (Dionisio-Sese and Tobita, 1998).

2. MATERIALS AND METHODS

2.1. Chemicals

Insecticides (Bifenthrin, Emamectin and Lembdacyhalthrin) were used to control *Helicoverpa armigera*. All of these chemicals were the products of Agro Mark Company, Pakistan.

2.2. Experiment 1

Two local varieties of pea *Pisum sativum L*. (Aleena Gold and Climax) were purchased from the local grain market of Faisalabad (Pakistan). The experiment was conceded at the experimental area, Department of Entomology, University of Agriculture, Faisalabad (Pakistan) by using factorial under Randomized Complete Block Design (RCBD). The total area of the plot was 32×14 meter. The total area was divided into small plots of 4.5×4.0 meter. The plants were treated with three insecticides i.e. Bifenthrin, Emamectin and Lambda cyhalothrin according to their field recommended doses. Three replicates of each experiment were conducted. The data of infestation reduction with these insecticides was collected after the 1st, 2nd and 3rd week of application. On the other handto check the efficacy of insecticides (Bifenthrin, Emamectin and Lambda cyhalothrin) on *H. armigera* population the same experiment was performed in the laboratory. Pea plants were sown in pots and a selected number of 1st instar larvae of *H.armigera* were released on each plant. After that, all the plants were covered with a net cloth so that the larvae do not move to another plant. All the plants were treated with each insecticide. Three replicates of each treatment were performed. The mortality percentage was observed after four and seven days of treatment.

2.3. Experiment 2

To evaluate the oxidative stress due to insecticides application leaves samples were collected weekly after the 1st, 2nd and 3rd week of insecticides treatment. The analysis of leaves samples was done to observe the antioxidant enzymes (CAT, SOD and POD) activities and non-enzymatic contents i.e. MDA or lipid peroxidation rate and proline contents in the plant after insecticide treatments. After this, to enhance the suppressed activities of antioxidant enzymes and non-enzymatic contents, a plant activator jasmonic acid (JA)

was applied exogenously and again analyzed the antioxidant enzymes (CAT, SOD and POD) activities and non-enzymatic contents i.e. MDA or lipid peroxidation rate and proline contents in the plant.

2.4. Extraction and determination of antioxidant enzymes activities

a) CAT activity

The enzyme mixture or sample was prepared by grounding fresh leaves (0.4 g) in 4 ml of extraction buffer containing (1% Triton X 100, 0.5 M Na-phosphate, pH 7.3, 3 mM EDTA,) and then centrifuged at 10,000 rpm for 10 minutes at 4 °C. Aebi (1984) technique with certain changes was used to determine CAT activity.

Chemicals required

- Phosphate buffer
- Hydrogen peroxide H_2O_2

Procedure

The CAT reaction solution having5.9 mM H_2O_2, 50 mM phosphate buffer (pH 7), and 0.1 ml enzyme extract which is our sample. The reaction was ongoing by pouring the sample (enzyme extract). Absorbance fluctuations were noted at 240 nm and read after every the 20s of the reaction solution. A single CAT unit action was cleared as per absorbance change of 0.01 units per minute.

b) SOD activity

The enzyme extract or sample was prepared by homogenizing fresh leaves (0.2 g) in 2.0 ml of extraction mixture containing 3 mM EDTA, 0.5 M Na-phosphate buffer, pH 7.3, 1% PVP, 1% Triton X-100 and then it was centrifuged at 10,000 rpm at 4 °C for 10 minutes. SOD activity was determined by applying Dhindsa's *et al.* (1981) process with a little amendment. To determined SOD activity, measured its capability by inhibiting photo reduction of nitrobluetetrazolium (NBT). The reaction solution contained 0.222 gm methionine, 0.0375 ml of Triton-X, 0.0132 gm of Riboflavin with 0.2 M buffer and 0.015 gm of NBT. All chemicals were added in 17.5 ml of distil H_2O. The enzyme mixture was contained on distil H_2O 800 µl, Phosphate buffer500 µl,Riboflavin100 µl, Methionine 200 µl, Triton-X 200 µl, NBT 100 µl, Sample 100 µl.

Procedure

Test tubes occupying with reaction mixture were set in UV light earlier including Riboflavin for 15 min.The absorbance of a mixture at 560 nm was resolved through spectrophotometer. A single unit of SOD activity had been characterized as the quantity of enzyme which hindered 50 % NBT photo reduction.

c) Peroxidase (POD) activity

The enzyme mixture or sample was prepared by grounding fresh leaves (0.4 g) in 4 ml of extraction buffer (1% Triton-X 100, 0.5 M Na-phosphate, pH 7.3, 3 mM EDTA). Afterward centrifuged at 10,000 rpm for 10 min. at 4°C.

Chemicals required

- Phosphate buffer
- Hydrogen per oxide H_2O_2
- Guaiacol

Procedure

The activity of peroxidase was calculated by the method of Liu *et al.* (2000) with a few adjustments. The POD reaction mixture having 0.1 ml enzyme extract, 50 mM phosphate buffer (pH 5), 40 mM H_2O_2 and 20 mM guaiacol. Fluctuations in absorbance in reaction mixture at 470 nm were resolved at each 20s. One unit of POD activity was described as an absorbance variation of 0.01 units min^{-1}.

(d) Estimation of Lipid peroxidation rate (MDA)

Oxidative damage to leaf lipids had been evaluated through substance of an aggregate of two thiobarbituric acid reactive substances (TBARS), stated as counterparts of malondialdehyde (MDA). The process of Cakmak and Horst (1991) was applied to measure the TBARS content in the leaves of *P. sativum* L. TBARS were used to quantify MDA levels (unit/ml).

Chemicals required

Distilled water, Sodium dodecyl sulfate (SDS), Acetic acid, n- butanol, Thiobarbituric acid (TBA).

Serum sample

1 ml of serum was taken and a 10% (w/v) homogenized mixture was prepared in 10 mM buffer having pH 7.4 and centrifuged at 13,000 rpm for 10 minutes at 4℃. The supernatant was utilized for quick TBARS.

Procedure

In the test tube, in 4.0 ml distilled water, we added1.5m ml of 0.8% TBA, 200 µl of 8.1% SDS,200μl of serum sample and 1.5 ml of 20% acetic acid solution (pH 3.5). And after that was warmed in a water bath at high temperature which was 90℃ for an hour. After heating, cooling was done under open water. 1.0 ml distill H_2O and 5.0 ml n-butanol had been included then it had been shaken forcefully and centrifuged at 4000 rpm for 10 minutes. The upper butanol layer had been taken and its absorbance was recorded at 532 nm.

(e) Proline determination

Chemicals required

Ninhydrin, Orthophosphoric acid, Sulfosalicylic acid and Glacial acetic acid.

Procedure

Proline from the dry leaf sample was appraised according to the technique of the Bates *et al.,* (1973). 0.5 g fresh leaf tissue was ground in 10 ml of 3 % sulfosalicylic acid and the extract was filtered through Whatman No. 2 filter paper. Then 2.0 ml of the filtrate was homogenized with 2.0 ml of acid ninhydrin solution containing (Ninhydrin 1.25 g) was mixed in 20 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid. After this, stored and cooled at 4 °C. After cooling, we added 2 ml of glacial acetic acid in a test tube. This mixture was incubated at 100 °C for an hour and then cooled in an ice bath. Finally, 4.0 ml of toluene were poured in solution and mixed forcefully by allowing a constant stream of air for 1-2 min. The chromosphere having toluene was aspirated from the aqueous phase, heated to room temperature and the absorbance was read at 520 nm using spectrophotometer (HITACHI, U2800) and toluene was taken as blank. The proline contents were determined on the dry weight basis as follows:

M mole proline g-1 fresh weight = (µgproline ml-1 x ml of toluene/115.5) / (g of sample)

3. RESULTS AND DISCUSSION

Legume foods is also used as a feed crop in various farming systems and get higher prices as compared to cereals and is progressively grown to additional farmers' incomes (Gowda *et al*., 1997). Pea seeds are the great mean of energy and protein for both animals and humans in all over the world (Savage and Deo, 1989). *Helicoverpa armigera* is an insatiable feeder pest which causes damage to more than 100 plant species including economically important and prevalent crops like cotton, tomato, chickpea, green pea, pigeon pea and maize etc. (Talekar *et al*. 2006). Many diverse conventional insecticides are used to the control of *H. armigera* which is harmful to vegetables (Wakchaure, 1998; Nadagouda *et al.*, 2010). This pest is mainly managed by synthetic insecticides. Bifenthrin and Cypermethrin have the great impact to control the population of pod *Helicoverpa armigera*on horticultural crops (Hara, 1986; Mujica *et al.*, 2000) and on vegetables (Civelek and Weintraub, 2003). Plants have natural anti-oxidative mechanism to prevent from hazardous effects of cellular membranes, produced by reactive oxygen species (ROS) (Zaefyzadeh *et al.*, 2009). Due to the stress condition, the plant may change the actions of ROS scavenging enzymes superoxidase dismutase (SOD), catalase (CAT) and peroxidase (POD) (Tuluce and Celik, 2006). Various physiological and metabolic processes in plants are regulated by plant growth regulators i.e. esters of JA, jasmonic acid and methyl jasmonate are naturally occurred (Keramat *et al*. 2009). Under both biotic and abiotic stresses, the increased contents of JA were reported in plant tissues.

Table 1 Comparison of mean % mortality of *H. armigera* after insecticides application at 4 and 7 post-spray days

Insecticides	Day 4	Day 7	Main effect
Bifenthrin	58.33±10.14^{b}	71.67±6.01^{ab}	65.00±6.68^{b}

Emamectin	65.00±5.00^{b}	78.33±1.66^{ab}	71.67±6.68^{b}
Lambdacyhalothrin	60.33±3.33^{b}	76.67±6.66^{ab}	68.50±7.70^{b}
Control	20.00±6.67^{c}	26.66±6.67^{c}	23.33±3.33^{c}
Main effect	49.16±10.01^{a}	63.33±7.34^{a}	

The exogenous application of JA showed a noticeable increase in CAT and SOD activities. Increased GR and APX activities were also reported but that was not significant as in SOD. It also decreased the oxidative stress by lowering the H_2O_2 and TBARS contents and increased the chlorophyll and carotenoids contents, growth parameters and membrane stability (Agarwal *et al*., 2005).

Table 1 represented the mean mortality of *H. armigera* at different post-spray intervals.It was observed that the maximum mortality was obtained by Emamectin i.e. 65% and 78% at four and seven days of treatment as compared to their control treatment. On chickpea, more effectiveness was observed in Emamectin treatment (Choudhary *et al*. 2005). Lambda cyhalothrin treatment gave 60% and 76% control of *H. armigera* after four and seven days of treatment. The lower population of larvae of *H. armigera* was obtained from Lambda cyhalothrin treatment (Shah *et al*. 2003). On the other hand minimum control was achieved by Bifenthrin treatment i.e. 58% and 71% after four and seven days of treatment.

Table 2 Comparison of mean % reduction in infestation *H. armigera* after insecticides application at 1st, 2nd and 3rd post-spray intervals

Insecticides	1st Week	2nd Week	3rd Week	Main effect
Bifenthrin	46.00±2.05^{cd}	42.00±4.20^{d}	38.00±3.53^{d}	42.00±3.08^{c}
Emamectin	67.00±2.00^{a}	62.33±2.85^{b}	57.33±2.64^{b}	62.22±3.22^{b}
Lambdacyhalothrin	52.00±2.00^{d}	48.33±2.02^{d}	45.00±2.40^{c}	48.44±0.51^{c}
Control	73.00±1.89^{a}	68.00±1.76^{a}	63.33±1.94^{b}	68.33±0.92^{a}
Main effect	59.5±2.76^{b}	55.16±3.20^{a}	50.19±1.83^{c}	

Table 2 represented the infestation reduction of *H. armigera* population in field condition after insecticides application at different post-spray intervals.So it was proved that Emamectin was more effective as compared to Bifenthrin and Lambda cyhalothrin.

Table 3 CAT activity variation (IU/mg of protein) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals

Insecticides	Aleena Gold (V1)			Climax (V2)			Main effect
	1st Week	2nd Week	3rd Week	1st Week	2nd Week	3rd Week	
Bifenthrin	87.86±0.72^{cde}	88.50±0.74^{cde}	85.38±0.39^{de}	86.14±1.12^{cde}	85.86±1.13^{de}	86.86±0.61^{ce}	86.76±0.85^{b}
Emamectin	86.36±1.49^{cde}	86.63±0.94^{cde}	85.43±1.01^{de}	90.60±0.37^{bcd}	88.50±0.47^{cde}	83.18±0.36^{e}	86.78±0.89^{b}
Lambdacyhalothrin	88.94±1.29^{cd}	88.09±0.76^{cde}	85.65±0.11^{de}	87.41±1.13^{cde}	85.51±0.85^{de}	86.41±0.39^{cde}	87.002±0.98^{b}
Control	97.02±1.29^{a}	94.54±0.74^{ab}	88.66±0.58^{cde}	98.26±1.68^{a}	100.0±0.96^{a}	91.42±0.91^{bc}	94.98±1.12^{a}

Main effect	90.04±1.35^{a}	89.44±1.12^{a}	86.27±0.49^{b}	90.60±1.51^{a}	89.97±1.82^{a}	86.97±0.92^{b}

Table 4 SOD activity variation (IU/mg of protein) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals

Insecticides	**Aleena Gold (V1)**			**Climax (V2)**			**Main effect**
	1st Week	**2nd Week**	**3rd Week**	**1st Week**	**2nd Week**	**3rd Week**	
Bifenthrin	67.83±2.80^{abc}	57.31±2.20^{def}	34.55±0.87^{jkl}	58.16±3.33^{def}	65.6±7.42^{bcd}	33.49±1.11^{kl}	52.83±2.87^{b}
Emamectin	67.69±1.56^{abc}	42.64±0.94^{ghij}	34.00±0.98^{jkl}	74.02±0.66^{a}	75.31±7.23^{a}	41.28±1.29^{hijk}	55.82±2.35^{a}
Lambdacyhalothrin	60.85±1.60^{cde}	55.89±1.13^{c}	28.83±1.44^{l}	65.57±2.99^{bcd}	67.47±8.4^{bc}	35.09±1.69^{ijkl}	52.28±2.03^{b}
Control	53.71±1.12^{ef}	44.08±4.35^{ghi}	31.29±1.04^{ijk}	54.03±0.62^{ef}	40.16±0.99^{fgh}	30.92±1.02^{fg}	48.69±1.83^{c}
Main effect	62.52±1.93^{a}	49.98±2.10^{b}	34.17±1.21^{d}	62.94±2.49^{a}	64.64±2.85^{a}	40.19±2.14^{c}	

Table 5 POD activity variation (IU/mg of protein) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals

Insecticides	**Aleena Gold (V1)**			**Climax (V2)**			**Main effect**
	1st Week	**2nd Week**	**3rd Week**	**1st Week**	**2nd Week**	**3rd Week**	
Bifenthrin	23.29±1.35^{ab}	15.95±1.42^{cdefg}	13.94±2.44^{cdefghi}	24.83 ±1.76^{a}	13.94±1.36^{cdefghi}	9.46±0.24^{hi}	16.71±1.48^{a}
Emamectin	16.45±0.49^{bcdef}	13.49±0.42^{cdefghi}	13.30±3.29^{defghi}	17.63±1.15^{abcde}	18.74±1.76^{abcd}	9.27±0.16^{fghi}	14.81±1.21^{ab}
Lambdacyhalothrin	20.56±0.61^{ab}	14.6±0.98^{ab}	13.01±1.49^{defghi}	14.36±1.15^{cdefghi}	15.25±1.29^{ab}	8.31±0.32^{fghi}	14.55±1.15^{b}
Control	15.26±0.59^{cdefgh}	11.31±0.58^{efghi}	9.96±1.36^{fghi}	10.25±0.33^{fghi}	8.77±0.43^{ghi}	7.33±0.24^{i}	10.48±0.78^{c}
Main effect	18.89±1.03^{a}	13.86±0.65^{c}	12.55±1.08^{c}	16.77±1.69^{ab}	14.17±1.21^{bc}	8.59±0.33^{d}	

Table 6 Lipid peroxidation rate variation (µl/ml) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals

Insecticides	**Aleena Gold (V1)**			**Climax (V2)**			**Main effect**
	1st Week	**2nd Week**	**3rd Week**	**1st Week**	**2nd Week**	**3rd Week**	
Bifenthrin	32.83±1.02^{cdefgh}	36.65±1.59^{bcd}	27.63±1.40^{ghi}	48.98±1.71^{a}	33.56±5.29^{ghi}	41.85±3.74^{bcdefg}	35.92±1.54^{b}

Emamectin	38.27 ±0.98^{bcdefg}	31.37± 1.31^{bcd}	33.92±0.85 ^{abcd}	43.90±0.7 5^{ab}	49.23±0. 17^{a}	30.63±0. 43^{fghi}	39.39± 1.76^{a}
Lambdacyhalothrin	44.52±0.8 6^{ab}	41.56± 3.45^{abcde}	23.12± 0.49^{hi}	36.15± 1.24^{bcdefg}	38.71±0. 67^{abcdef}	35.36±0. 39^{bcdefg}	36.57± 1.24^{ab}
Control	36.23± 0.65^{bcdefg}	30.05± 1.35^{fghi}	20.63±0.46^{i}	31.76± 1.92^{defgh}	29.63±3. 63^{fghi}	31.06±0. 93^{efghi}	28.89± 1.46^{c}
Main effect	37.96± 1.34^{ab}	34.91± 1.65^{b}	28.57± 2.64^{c}	40.19± 2.11^{a}	36.28±2. 93^{ab}	34.72±1. 59^{b}	

Table 7 Proline contents variation (µg/ml) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals

Insecticides	**Aleena Gold (V1)**			**Climax (V2)**			**Main effect**
	1st Week	**2nd Week**	**3rd Week**	**1stWeek**	**2ndWeek**	**3rd Week**	
Bifenthrin	23.29± 1.35^{ab}	15.95± 1.42^{cdefg}	13.94± 2.44^{cdefghi}	24.83± 1.76^{a}	13.941.36 ^{cdefghi}	9.46±0.2 4^{hi}	16.71± 1.48^{a}
Emamectin	16.45± 0.49^{bcdef}	13.49± 0.42^{cdefghi}	13.30± 3.29^{defghi}	17.63± 1.15^{abcde}	18.74±1. 76^{abcd}	9.27± 0.16^{fghi}	14.81± 1.21^{ab}
Lambdacyhalothrin	20.56± 0.61^{ab}	14.69± 0.98^{ab}	13.01± 1.49^{defghi}	14.36± 1.15^{cdefghi}	15.25±1. 29^{ab}	8.31± 0.32^{fghi}	14.55± 1.15^{b}
Control	15.26±0.5 9^{cdefgh}	11.31± 0.58^{efghi}	9.96± 1.36^{fghi}	10.25± 0.33^{fghi}	8.77± 0.43^{ghi}	7.33± 0.24^{i}	10.48± 0.78^{c}
Main effect	18.89± 1.03^{a}	13.86± 0.65^{c}	12.55± 1.08^{c}	40.19± 2.11^{a}	36.28±2. 93^{ab}	34.72±1. 59^{b}	

Table 3 represented that CAT activity variation at different post-spray intervals. Deceased CAT activity was observed due to Bifenthrin treatment in both the varieties in all post-spray intervals especially in 2nd week as compared to its control treatment. Decreased CAT activity caused by insecticide stress was observed (Bashir *et al.* 2007; Parween *et al.* 2011). Table 4 represents that SOD activity variation at different post-spray intervals. Increased SOD activity was also observed due to Bifenthrin treatment in both the varieties in all three weeks especially in 2nd week as compared to its control treatment. Greater SOD activity was studied in *Glycin max* L. caused by insecticide stress (Bashir *et al.* 2007; Parween *et al.* 2011). Table 5 represents that POD activity variation at different post-spray intervals.

Greater POD activity was caused by insecticide stress in leaves of both pea varieties due to Emamectin treatment as compared to its control treatments. Maximum POD activity was recorded in V1 after 2nd week and in V2 after the 3rd week. Enhanced POD activity eliminated by increased membrane injury and Ca_2^+ concentrations was caused by salt stress (Gossett *et al.,* 1994). Table 6 shows lipid peroxidation rate variation (µl/ml) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals. Increased lipid peroxidation rate was observed in V1 due to Lambda cyhalothrin treatment after 2nd week and in V2 maximum lipid peroxidation rate was observed due to Emamectin and Bifenthrin in 1st and 2nd week.

The lipid peroxidation was commonly said by the increase in TBARS content of membrane. Cell membrane's injury by organophsphates, lipid peroxidation was the first step (Hazarika*et al.,* 2003). Deltamethrin and different other insecticides caused an increase in TBARS contents in *Glycine max* L. leaves (Bashir *et al.* 2007; Song *et al.* 2007). Table 7 shows proline contents variation (µg/ml) in *Pisum sativum* L. in response to different insecticides application at different post-spray intervals. The increased proline contents were observed due to Bifenthrin treatment in V1 after all three weeks but maximum after 2nd week then decrease in the 3rd week. In V2, the greater proline contents were also observed in Bifenthrin treatment and increased with age after 2nd week. Increase in proline contents was expressed due to insecticide stress in Vigna *radiate* L (Parween *et al.* 2012), in *Glycine max* L. (Bashir *et al.* 2007).

4. CONCLUSION

The current experiment evaluates the responses of *Pisum sativum* L. against three different insecticides including Bifenthrin, Emamectin and Lambda cyhalothrin, also elucidated the capability of pesticide metabolizing anti-oxidative enzyme system. Triggering of metabolic processes in plant cells in response to chemical stress is demonstrated in (i) accretions of proline, and (ii) up rise in numerous enzymatic and non-enzymatic antioxidants in many plant parts, thus signifying that effectiveness of Asc–Glu cycle increases as to detoxify ROS in cells. Likewise, it exposed the convoluted indication about breakdown of insecticides molecules by the greater action position of oxidoreductase enzymes. Such biochemical outcomes can be understood as internal tolerance mechanisms and may permit us to make improved approaches for decreasing the risks of insecticide adulteration in crop production. Enzyme expression at the gene level and their breakdown studies by identifying intermediate degradation compounds are the problems for future concerns.

REFERENCE

1. Aebi, H., 1984. Catalase in vitro. J. Methods Enzymol., 105: 121–126.
2. Agarwal, S., R. Sairam, G. Srivastava and R. Meena. 2005. Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. J. Biol. Plant.. 49: 541-550.
3. Ahmed, K., A.S. Qureshi and F. Khalique, 2000.Effect of environmental factors on pheromone trap catches of chickpea pod borer, *Helicoverpa armigera*(Hub.) from 1983 to 1998. Proc. Pak. Acad. Sci., 37: 227–38.
4. Ambrose, M.J., 1995. From Near East Centre of origin the prized pea migrates throughout world diversity. J., Appl. Ecol., 9: 67-77.
5. Anwar, M. and M. Shafique, 1993.Integrated control of gram pod borer, *Helicoverpa armigera* (Hubner) in Sindh. Proc. Pak. Congr. Zool., 215-222 pp.
6. Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. J. Biotechnol. adv., 27: 84-93.
7. Ashraf, M., (2004).Some important physiological selection criteria for salt tolerance in plants. J. Flora., 199: 361–376.
8. Bashir, F., T. Siddiqi, I.M.M. Zafar and M. Iqbal. 2007. Effect of different concentrations of mancozeb on the morphology and anatomy of *Lens culinaris* L. Indian J. Environ. Sci. 11: 71-74.
9. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. J. Plant Soil, 39: 205–207.
10. Bray, E.A., J. Bailey-Serres and E. Weretilnyk. 2000. Responses to abiotic stresses. J. Biochem. Mol. Boil. Plants., 1158-1203.
11. Breeden, D.C., T.E. Young, R.M. Coates and J.A. Juvik. 1996. Identification and bioassay of kairomones for Helicoverpazea. J. Chem. ecol. 22: 513-539.
12. Cakmak, I. and W.J. Horst, 1991.Effect of aluminium on lipid peroxidation, superoxide dismutase catalase and peroxidase activities in root tips of soybean (*Glycine max* L.). J. Physiol. Plant. 83: 463–468.
13. Chaudhry, M.T., M.A. Malik and N. Iqbal. 2005. Management of *Helicoverpa armigera* with different insecticides. Pak. J. Agri. Sci., 42: 1-2.
14. Chen, C.T. and C.H. Kao. 1993. Osmotic stress and water stress have opposite effects on putrescine and proline production in excised rice leaves. Plant Growth Regul., 13: 197-202.
15. Civelek, H.S. and P.G. Weintraub, 2003. Effects of bensultap on larval pod borer *Helicoverpa armigera*(Lepidoptera: Noctuidea) in tomatoes. J. Crop. Prot., 22: 479-483.
16. Dhindsa, R.S., D.P. Plumb and T.A. Thorpe, 1981. Leaf senescence correlated with increased level of membrane permeability, lipid peroxidation and decreased level of SOD and CAT. J. Exp. Bot., 32: 93–101.
17. Dionisio-Sese, M.L. and S. Tobita, 1998. Antioxidant responses of rice seedlings to salinity stress. J. Plant Sci., 135: 1–9.
18. Dowling, D.K. and L.W. Simmons. 2009. Reactive oxygen species as universal constraints in life history evolution. Proc. R. Soc. Part B, Biol. Sci., 276: 1737–1745.
19. Eger, J.R.J.E. and L.B. Lindenberg, 1998.Utility of spinosad for insect control in Florida vegetables.Proc. Fla. State Hortic. Soc., 111: 55-57.
20. Faize, M., L. Burgos, L. Faize, A. Piqueras, E. Nicolas, G. Barba-Espin, M.J. Clemente-Moreno, R. Alcobendas, T. Artlip and J.A. Hernandez, 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. J. Exp. Bot., 62: 2599-2613.
21. Fathipour, Y. and B. Naseri 2011.Soybean Cultivars Affecting Performance of*Helicoverpa armigera*(Lepidoptera: Noctuidae). J. Plant. Sci. 6: 124-129.
22. Ferguson, J.S., 2004. Development and stability of insecticide resistance in the pod borer *Helicoverpa armigera*to abamectin and spinosad. J. Econ. Entomol., 97(1): 112-119.
23. Gatel, F. and F. Grosjean (1990). Composition and nutritive value of peas for pigs: a review of European results. Livest.Produc. Sci., 26: 155±17.
24. Hazarika, A., S. Sarkar, S. Hajare, M. Kataria and J. Malik. 2003. Influence of malathion pretreatment on the toxicity of

anilofos in male rats: a biochemical interaction study. J. Toxicol., 185: 1-8.
25. Keramat, B., K.M. Kalantari and M.J. Arvin. 2009. Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (Glycine max L.). Afr. J. Microbiol Res., 3: 240-244.
26. Lee, J.H., W.T. Kim, 2011. Regulation of abiotic stress signal transduction by E3 ubiquitin ligases in Arabidopsis. J. Mol. Cells., 31: 201–208.
27. Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant Physiol., 148: 4–14.
28. Lin, C.L. and C.H. Kao, 2002. Osmotic stress-induced changes in cell wall peroxidase activity and hydrogen peroxide level in roots of rice seedlings. J. Plant Growth Regul., 37: 177–184.
29. Liu, B., J.-W.Jiang, B.C. Wilson, L. Du, S.-N.Yang, J.-Y.Wang, G.-C.Wu, X.-D.Cao and J.-S.Hong. 2000. Systemic infusion of naloxone reduces degeneration of rat substantianigral dopaminergic neurons induced by intranigral injection of lipopolysaccharide. J. Pharmacol. Exp. Ther., 295: 125-132.
30. Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. J. Trends Plant Sci., 7: 405-410.
31. Mujica, N., M. Pravatiner, and F. Cisneros, 2000. Effectiveness of abamectin and plant - oil mixtures on eggs and larvae of the leaf miner fly, *Liriomyzahuidobrensis* Blanchard. CIP Program Report 1999- 2000: 161- 166.
32. Nadagouda, S., B.V. Patil and S.A.G. Venkateshalu, 2010.Studies on development of resistance in serpentine pod borer *Helicoverpa armigera*(Lepidoptera: Noctuidea) to insecticides. Karnataka J. Agric. Sci., 23:56-58.
33. Nawkar, G.M., P. Maibam, J.H. Park, V.P. Sahi, S.Y. Lee and C.H. Kang. 2013. UV-induced cell death in plants. Int. J. Mol. Sci., 14: 1608–1628.
34. Nicholas, J. and J.S. Wood. 2001. Catalase and superoxide dimutase activity in ammonia-oxidising bacteria. J. FEMS Microbiol. Ecol., 38: 53-58.
35. Parween, T., S. Jan and T. Fatma. 2012. Evaluation of oxidative stress in *Vignaradiata* L. in response to chlorpyrifos. Int. J. Environ. Sci. Technol., 9: 605-612.
36. Ruan Y.M. and K.J. Wu, 2001.Performances of the cotton bollworm, *Helicoverpa armigera*on different food plants. J. ActaEntomol.Bohemos.,44: 205-212.
37. Savage, G. and S. Deo. 1989. The nutritional value of peas (Pisum sativum). A literature review.Nutr.Abstr. Rev., (Series A): 66-88.
38. Shah, Z.A., M.K. Shahzad and M.A. Sharaz. 2003. Efficacy of different insecticides against larval population density of gram pod borer, *Helicoverpa armigera* (Hub.) with reference to chickpea in Faisalabad–Pakistan. Int. J. Agri. Biol. 5:326-328.
39. Sharma, H. 2001. Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera): biology and management. J. Crop Pro. Comp., 72.
40. Sharma, H.C., P.C. Stvenson and C.L.L. Gowada, 2005.*Heliothis/Helicoverpa* management: emerging trends and strategies for future research. Oxford, IBH Pub. Co. Pvt. Ltd.
41. Singh, A. and S. Mullick. 2002. Leaf volatiles as attractants for neonate *Helicoverpa armigera* (Lepedoptera: Noctuidae) larvae. J. Appl. Entomol. 126: 14-19.
42. Singh, I. and N. Saravanan, 2011.Early Eocene warming events and the timing of terrestrial faunal exchange between India and Asia. J. Geol., 39:15-18.
43. Singh, O. and S. Parihar. 1988. Effect of different hosts on the development of *Heliothisarmigera* Hub. J. Bull. Entomol. Res., 29: 168-172.
44. Song, N.H., X. Le Yin, G.F. Chen and H. Yang. 2007. Biological responses of wheat (*Triticumaestivum*) plants to the herbicide chlorotoluron in soils. J. Chemos., 68: 1779-1787.
45. Talekar, N., R. Opena and P. Hanson. 2006. Helicoverpa armigera management: a review of AVRDC's research on host plant resistance in tomato. J. Crop Pro., 25: 461-467.
46. Timmerman-Vaughan, G.M., A. Mills, C. Whitfield, T. Frew, R. Butler, S. Murray, M. Lakeman, J. Mccallum, A. Russell and D. Wilson. 2005. Linkage mapping of QTL for seed yield, yield components, and developmental traits in pea. J. Crop Sci., 45: 1336-1344.
47. Tuluce, y. and I. Celik, 2006. Influence of subacute treatment of some plant growth regulators on serum marker enzymes and erythrocyte and tissue antioxidant defense and lipid peroxidation in rats. J. Biochem. Mol. Toxicol., 20(4): 174-182.
48. Urbano, G., P. Aranda, A. Vilchez, C. Aranda, L. Cabrera, J.M. Porres and M.L. Jurado, 2005. Effects of germination on the composition and nutritive value of proteins in *Pisum sativum L*. J. Food Chem., 93: 671-679.
49. Venette, R. C., E. E. Davis, J. Zaspel, H. Heisler, and M. Larson. 2003. Mini Risk Assessment Old World bollworm, *Helicoverpa armigera*Hübner (Lepidoptera: Noctuidae). American J. of Exp. Biol., 6: 1776-1778.
50. Wakchaure, B. M., 1998. Life history and control of tomato borer, *Helicoverpa armigera*(Lepidoptera: Noctuidea). M.Sc. (Agric.) thesis submitted to Mahatma PhuleKrishVidyapeeth, Rahuri.
51. Westerfield, 2013.Food Plot Species Profile: Austrian Winter Peas. Annual report of crop production Australia.
52. Yildiztekin M., C. Kaya, A. L. Tuna and M.Ashraf, (2015). Oxidative stress and antioxidative mechanisms in tomato (*Solanumlycopersicum L.*) plants sprayed with different pesticides. Pak. J. Bot., 42(2): 717–721.
53. Zaefyzadeh, M.V., R.A. Quliyev, S. Babayeva and M.A. Abbasov, 2009.The effect of the interaction between

genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces.Turk. J. Biol., 33: 1-7.

54. Zhang, H., F. Zhang, Y. Xia, G. Wang and Z. Shen. 2010. Excess copper induces production of hydrogen peroxide in the leaf of Elsholtziahaichowensis through apoplastic and symplastic CuZn-superoxide dismutase. J. Hazard. Mater., 178: 834-843.
55. Zohary, D. and M. Hopf, 2000.Domestication of Plants in the Old World. Oxford University Press: Oxford, UK.